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L'IMPACT DE L'ENVIRONNEMENT ET DE L'ÉPIGÉNÉTIQUE DANS DES MALADIES INFLAMMA-TOIRES

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CEA-Institute de Biologie François Jacob

13th March 2018

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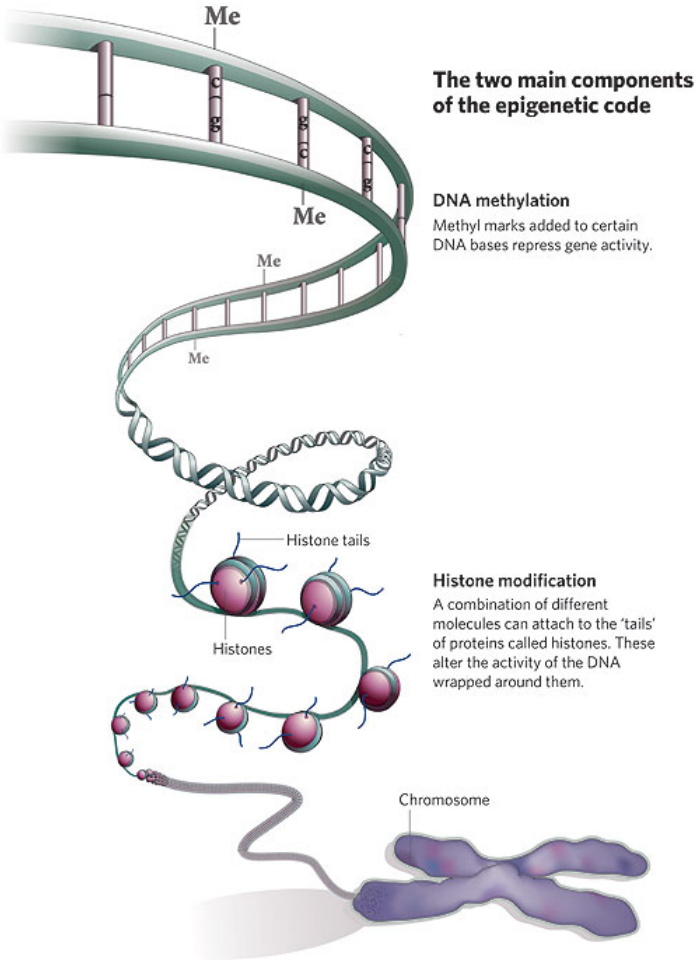
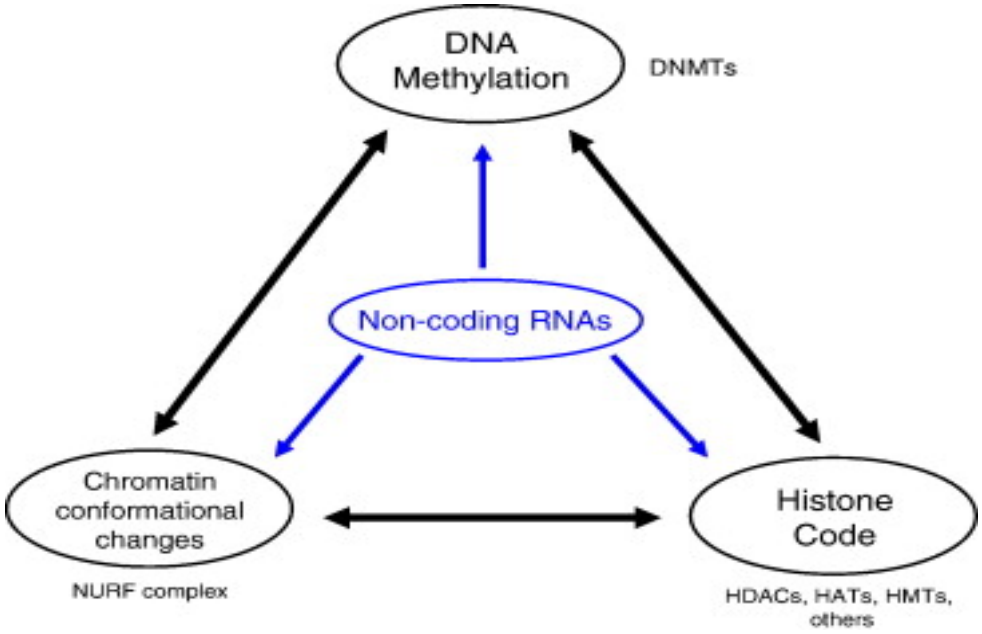
EPIGENETICS – THE REGULATORY PLATFORM OF A CELL

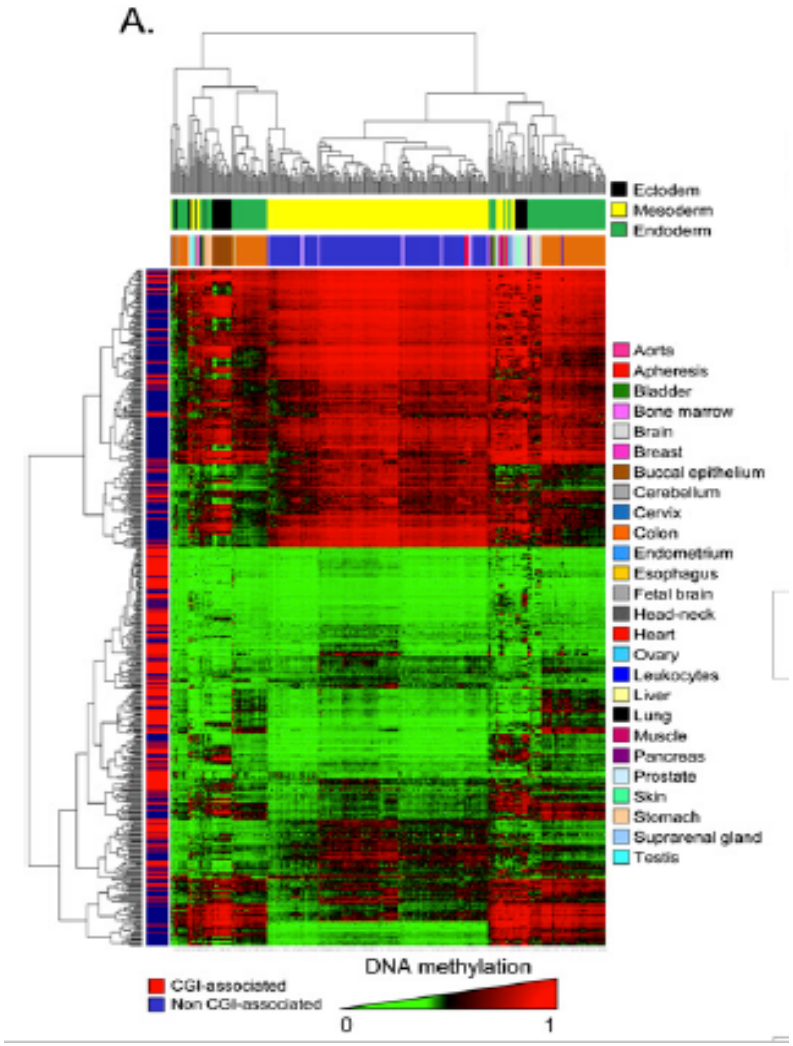


Qiu, Nature, 2006

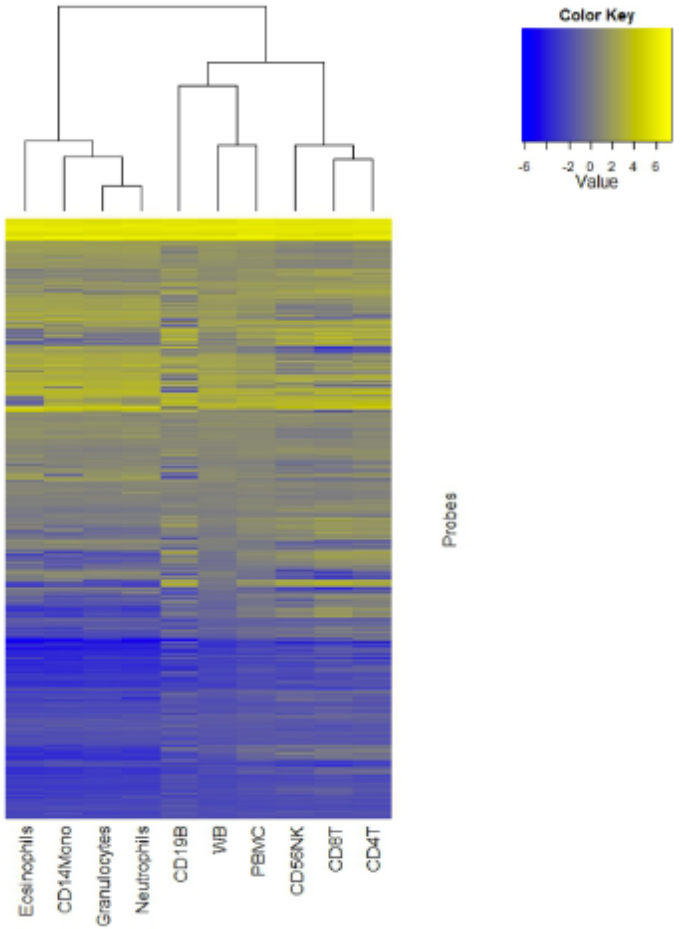
Costa, Gene, 2008



DNA METHYLATION PROFILES ARE TISSUE SPECIFIC



Fernandez et al., Genome Res., 2012



Reinius et al., PLoS ONE., 2012

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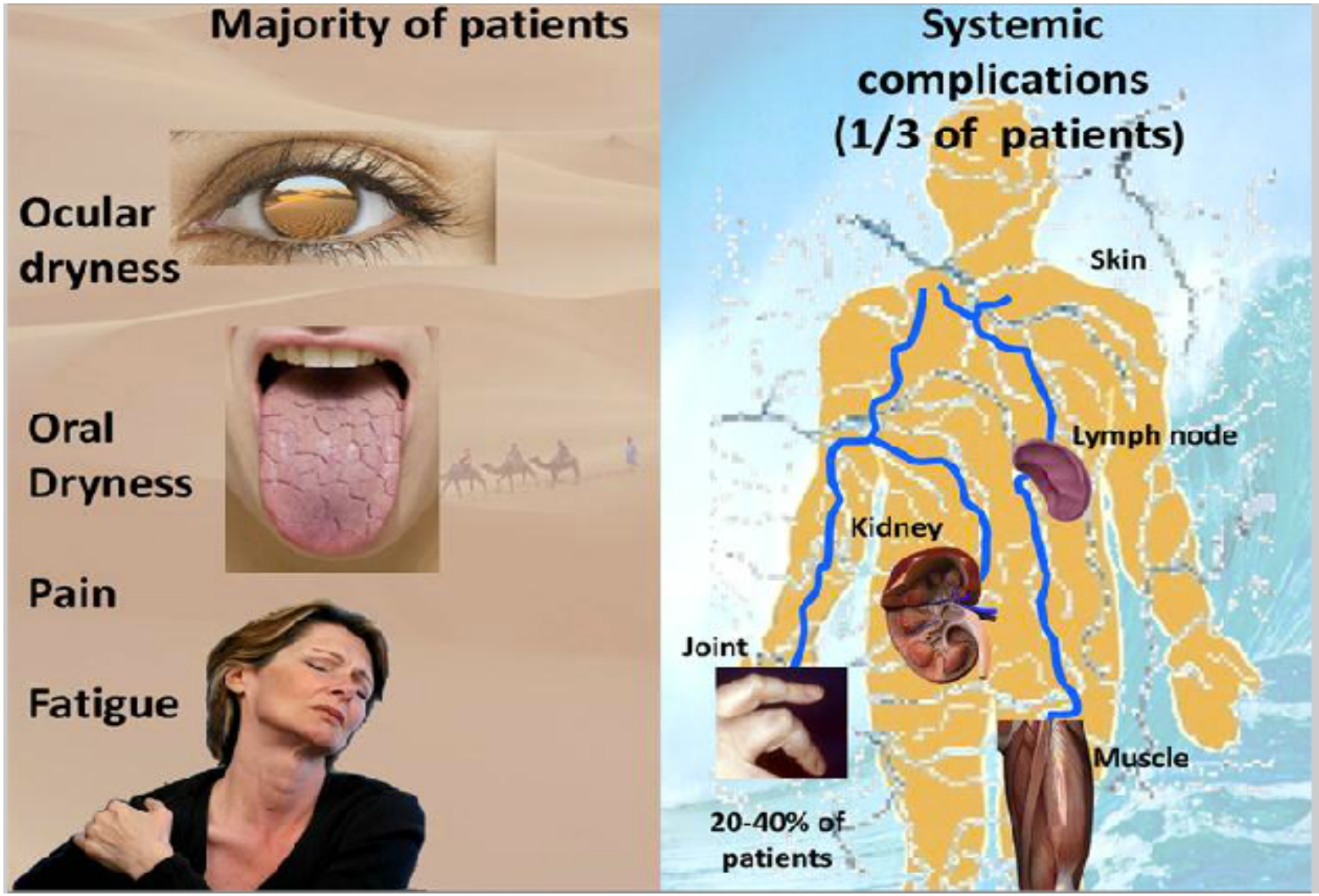
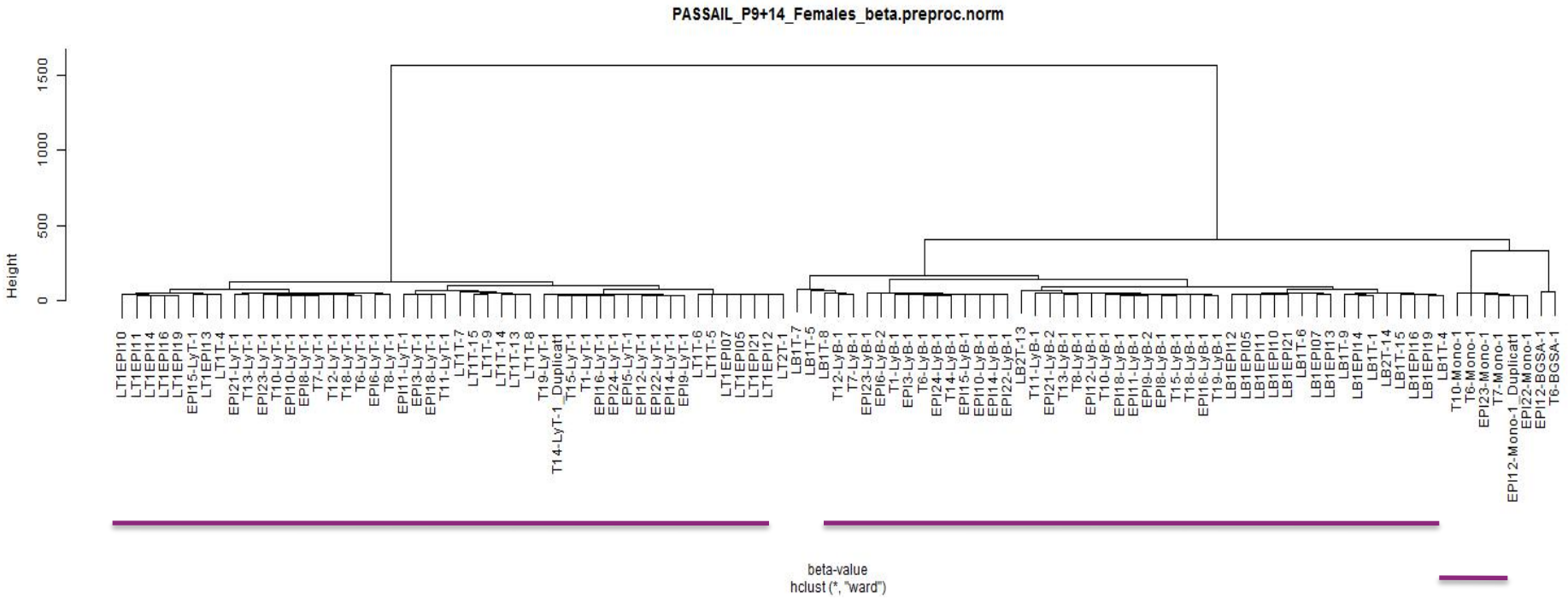
PRIMARY SJÖGREN'S SYNDROME



CLUSTERING OF THE DATA



T lymphocytes

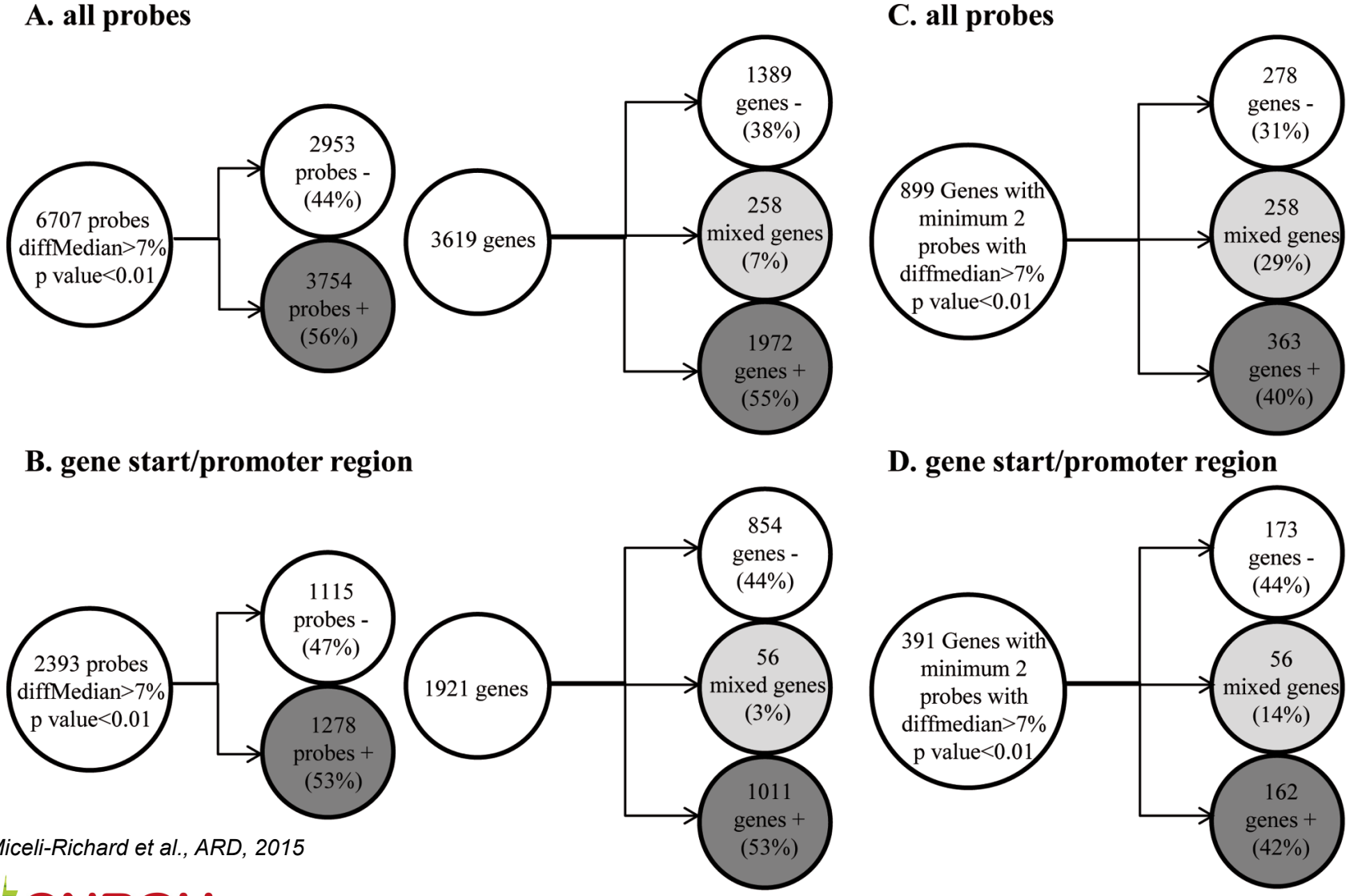
B lymphocytes

Monocytes

Epithelial cells from salivary glands

Miceli-Richard et al., ARD, 2015

MORE METHYLATION CHANGES IN B CELLS COMPARED TO T CELLS IN PSS



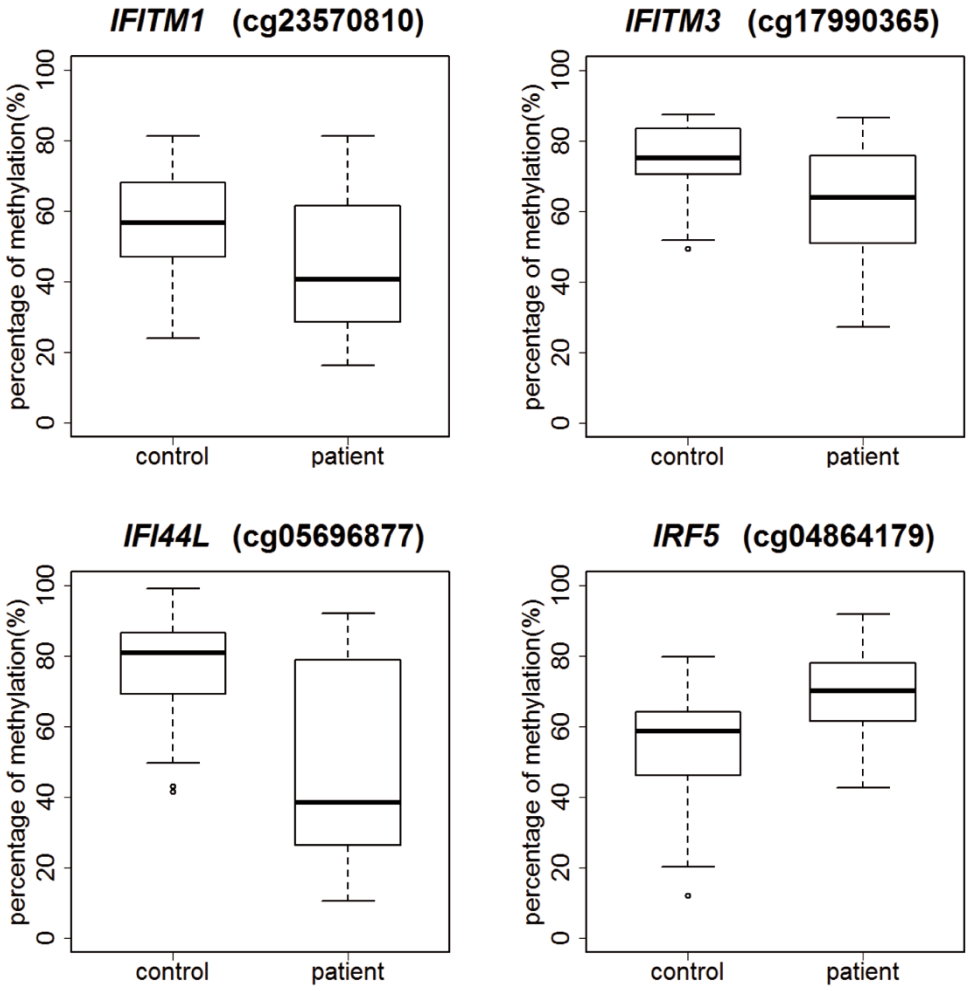
Miceli-Richard et al., ARD, 2015

VALIDATION OF DNA METHYLATION CHANGES IN INTERFERON REGULATD GENES

A.

Genes	probes used in 450K found significantly changed in this study	p value/ pyrosequencing
IFITM1	cg23570810	4.00e-02
IFITM3	cg17990365	2.31e-02
IFI44L	cg03607951	9.63e-05
	cg17980508	9.42e-07
	cg05696877	4,59E-04
IRF5	cg04864179	1.35e-03
RUNX3	cg24019564	1.80e-05
	cg09993145	1.92e-03
TNFAIP8	cg14692284	2.50e-04
	cg03665078	4.87e-04
IKZF1	cg09241714	3.36e-04
	cg17157198	8.82e-05
	cg07621224	4.24e-04
SLC15A4	cg24301247	8.08e-05
GRB2	cg06943385	7.56e-04
MIR21	cg04276626	1.38e-03
IL21R	cg00050618	1.12e-03
TRAF5	cg10177528	2.99e-04
CXCR5	cg04537602	4.95e-04
	cg13298528	2.93e-05
	cg19791714	7.76e-04

B.



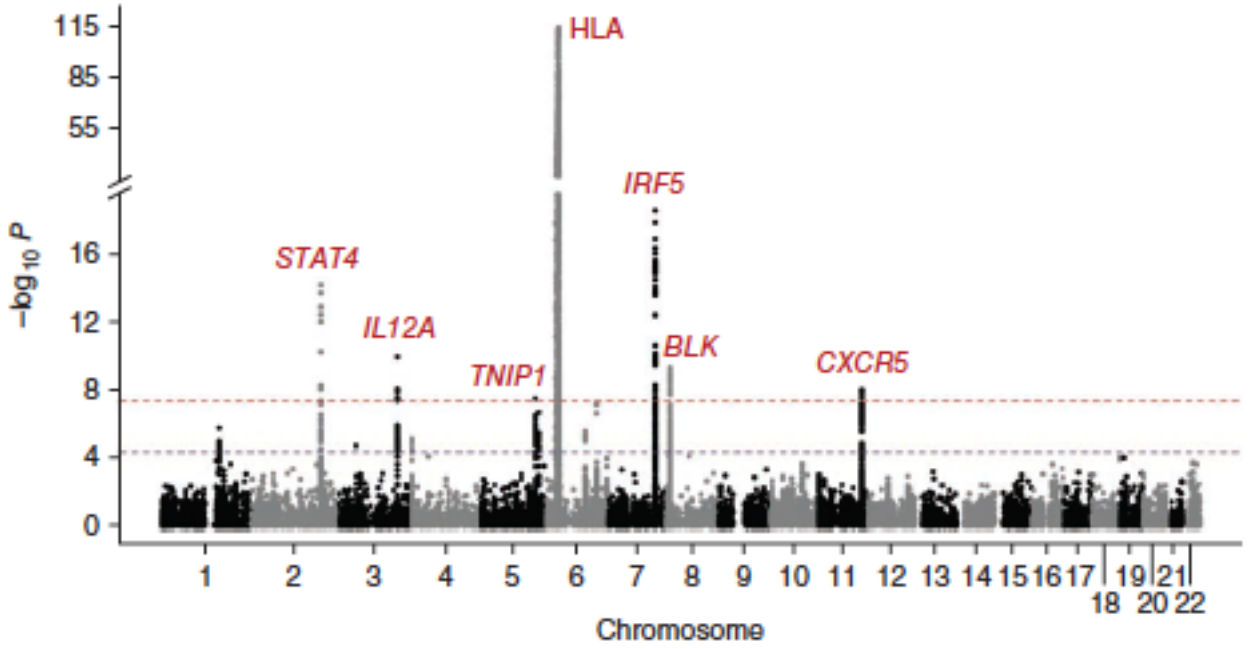
Miceli-Richard et al., ARD, 2015

GWAS REGIONS IN SJOGREN'S SYNDROME

nature genetics

Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjögren's syndrome

Christopher J Lessard[1,2], He Li[1,2], Indra Adrianto[1], John A Ice[1], Astrid Rasmussen[1], Kiely M Grundahl[1],



GWAS LOCI ARE ALSO ENRICHED IN THE METHYLATION DATA

Table 1 Top associations in the HLA region

SNP	Alleles[a]	MAF[b] Case	MAF[b] Control	Observed (O) or Imputed (I) in DS1/DS2	DS1 OR[c] (95% CI)	DS1 P	DS2 OR[c] (95% CI)	DS2 P	Meta-analysis OR[c] (95% CI)	Meta-analysis P
HLA-DRA										
rs112357081	TCTAA/T	0.59	0.33	I/–	2.89 (2.43–3.44)	1.01×10^{-22}	–	–	–	–
rs3135394	A/G	0.27	0.11	I/O	3.52 (2.83–4.38)	2.47×10^{-29}	3.52 (3.10–3.99)	1.14×10^{-85}	3.52 (3.02–4.10)	5.22×10^{-113}
HLA-DQB1										
rs115575857	A/G	0.29	0.12	I/I	3.25 (2.61–4.05)	4.10×10^{-26}	3.65 (3.22–4.14)	3.70×10^{-90}	3.53 (3.03–4.11)	7.65×10^{-114}
rs3129716	T/C	0.29	0.12	I/O	3.29 (2.66–4.09)	2.11×10^{-27}	3.51 (3.10–3.97)	7.74×10^{-87}	3.45 (2.97–4.00)	4.59×10^{-112}
HLA-DQA1										
rs116232857	A/G	0.64	0.42	I/I	2.83 (2.37–3.38)	9.05×10^{-21}	2.42 (2.19–2.67)	1.14×10^{-67}	2.53 (2.24–2.86)	1.33×10^{-96}
rs9271588	T/C	0.27	0.48	I/O	0.35 (0.29–0.42)	1.93×10^{-28}	0.43 (0.39–0.48)	4.62×10^{-69}	0.41 (0.36–0.46)	1.37×10^{-85}

[a]Major allele/minor allele. [b]The minor allele frequency (MAF) was calculated using combined DS1 and DS2, except for rs112357081, for which the MAF was derived from DS1 only. [c]ORs for each variant indicate the disease risk conferred by the minor allele.

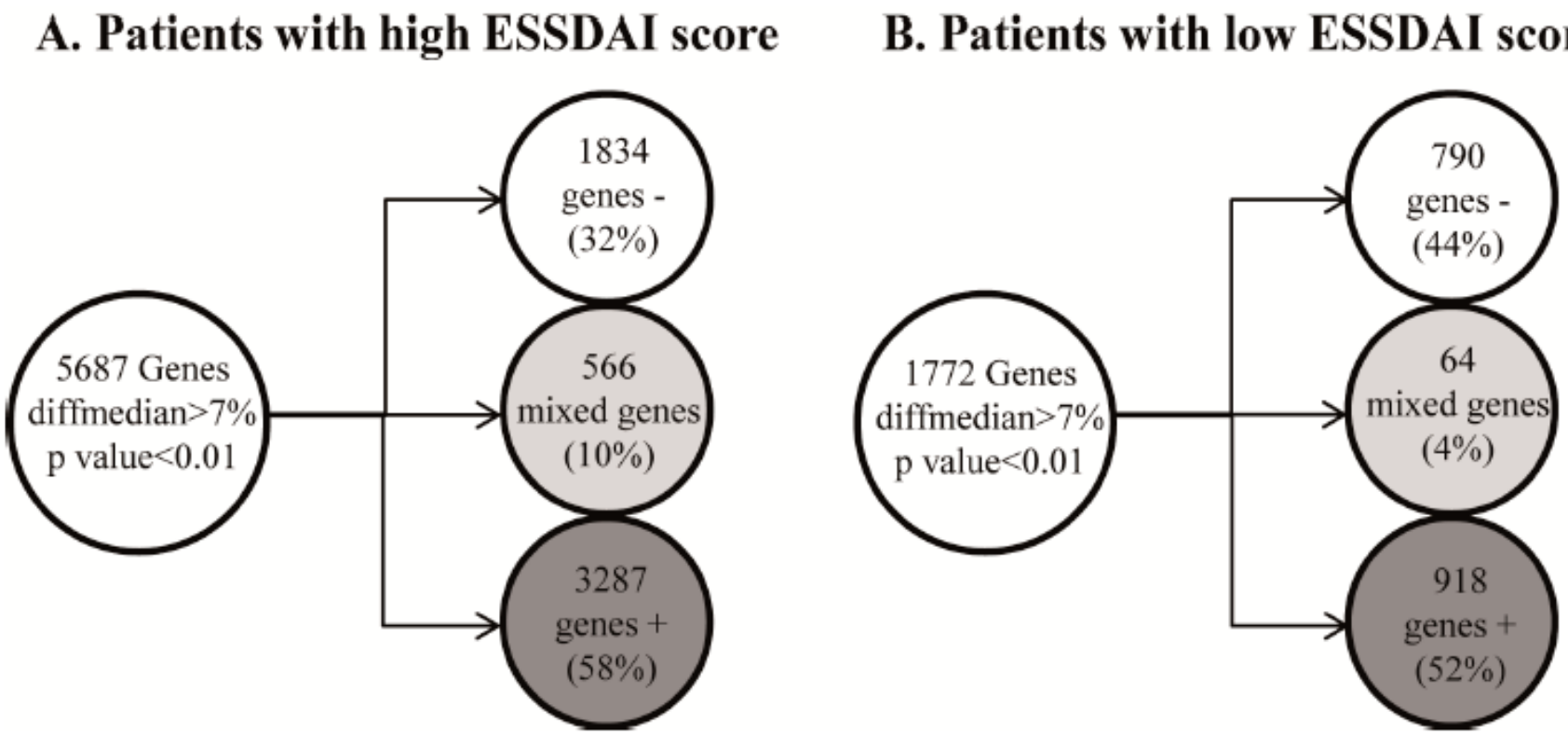
Table 2 Non-HLA regions associated with Sjögren's syndrome at GWS

SNP	Alleles[a]	MAF[b] Case	MAF[b] Control	Observed (O) or Imputed (I) in DS1/DS2	DS1 OR[c] (95% CI)	DS1 P	DS2 OR[c] (95% CI)	DS2 P	Meta-analysis OR[c] (95% CI)	Meta-analysis P
IRF5 **(chr7)**										
rs3757387	T/C	0.54	0.45	I/I	1.31 (1.11–1.54)	1.49×10^{-3}	1.50 (1.37–1.64)	7.50×10^{-18}	1.44 (1.29–1.62)	2.73×10^{-19}
rs4728142	G/A	0.53	0.44	O/O	1.29 (1.09–1.52)	2.85×10^{-3}	1.46 (1.33–1.60)	2.05×10^{-15}	1.40 (1.25–1.57)	9.75×10^{-17}
rs17339836	C/T	0.18	0.12	O/O	1.65 (1.32–2.06)	8.53×10^{-6}	1.55 (1.37–1.75)	5.73×10^{-12}	1.58 (1.36–1.84)	2.43×10^{-16}
rs17338998	C/T	0.18	0.12	I/I	1.64 (1.32–2.05)	9.51×10^{-6}	1.55 (1.37–1.75)	5.70×10^{-12}	1.57 (1.35–1.83)	2.67×10^{-16}
rs10954213	A/G	0.34	0.38	O/O	0.83 (0.70–0.98)	2.91×10^{-2}	0.82 (0.74–0.90)	3.63×10^{-5}	0.82 (0.73–0.92)	3.20×10^{-6}
STAT4 **(chr2)**										
rs10553577	TATA/T	0.30	0.23	I/I	1.38 (1.15–1.65)	5.13×10^{-4}	1.45 (1.31–1.61)	2.30×10^{-12}	1.43 (1.26–1.62)	6.80×10^{-15}
rs13426947	G/A	0.24	0.19	O/O	1.34 (1.11–1.62)	2.44×10^{-3}	1.31 (1.18–1.47)	1.09×10^{-6}	1.32 (1.16–1.51)	9.45×10^{-9}
[illegible]										
rs485497	G/A	0.54	0.48	O/O	1.32 (1.12–1.55)	9.47×10^{-4}	1.30 (1.18–1.42)	3.15×10^{-8}	1.30 (1.16–1.46)	1.17×10^{-10}
rs583911	A/G	0.48	0.42	I/I	1.29 (1.10–1.52)	2.16×10^{-3}	1.26 (1.15–1.38)	1.28×10^{-6}	1.27 (1.13–1.42)	9.88×10^{-9}
BLK **(chr8)**										
rs2736345	A/G	0.36	0.29	I/I	1.16 (0.97–1.37)	1.01×10^{-1}	1.37 (1.24–1.50)	2.76×10^{-10}	1.30 (1.16–1.47)	4.97×10^{-10}
rs2729935	C/A	0.41	0.35	I/I	1.28 (1.08–1.52)	4.02×10^{-3}	1.30 (1.19–1.43)	4.29×10^{-8}	1.30 (1.16–1.46)	6.85×10^{-10}
[illegible]	G/A	0.37	0.32	O/I	1.34 (1.13–1.59)	6.75×10^{-4}	1.23 (1.12–1.36)	2.65×10^{-5}	1.26 (1.12–1.42)	7.96×10^{-8}
CXCR5 **(chr11)**										
rs711[illegible]038	A/G	0.18	0.23	I/I	0.79 (0.64–0.98)	3.32×10^{-2}	0.72 (0.64–0.81)	6.33×10^{-8}	0.74 (0.64–0.86)	1.10×10^{-5}
rs493[illegible]443	T/C	0.16	0.20	O/O	0.79 (0.63–0.98)	3.21×10^{-2}	0.74 (0.65–0.83)	5.03×10^{-7}	0.75 (0.65–0.87)	6.82×10^{-6}
[illegible]										
rs6579837	G/T	0.12	0.09	I/I	1.58 (1.23–2.04)	3.94×10^{-4}	1.38 (1.19–1.59)	1.71×10^{-5}	1.43 (1.20–1.71)	3.30×10^{-8}
rs7732451	A/G	0.15	0.12	O/O	1.36 (1.09–1.71)	6.77×10^{-3}	1.33 (1.17–1.52)	2.43×10^{-5}	1.34 (1.14–1.57)	5.32×10^{-7}

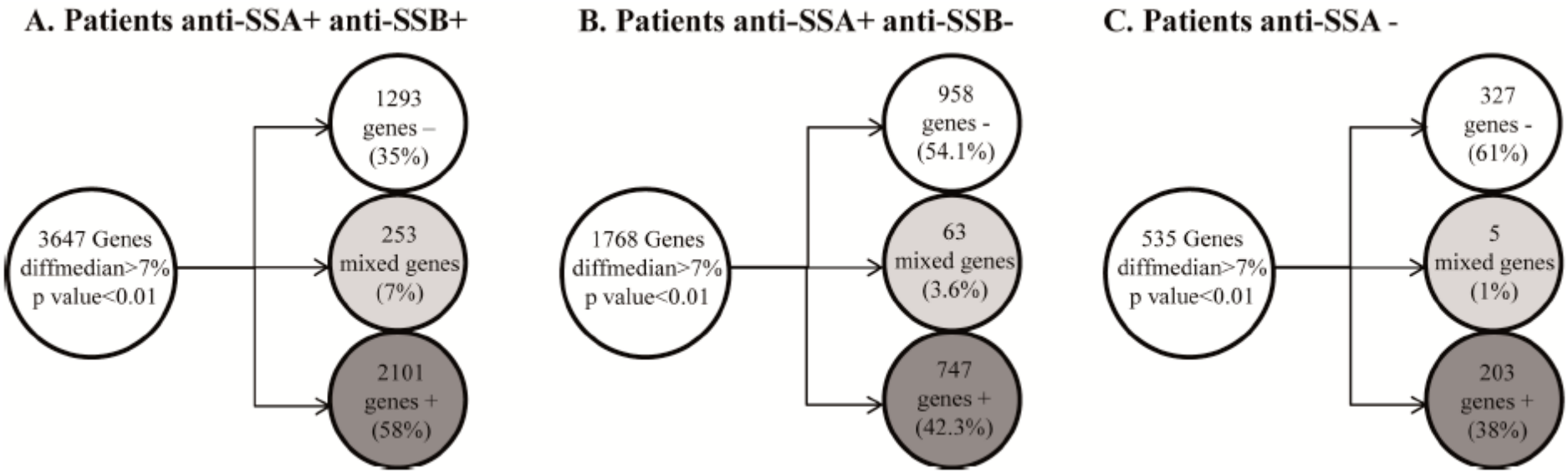
[a]Major allele/minor allele. [b]The MAF was calculated using combined DS1 and DS2. [c]ORs for each variant indicate the disease risk conferred by the minor allele.

Miceli-Richard et al., ARD, 2015

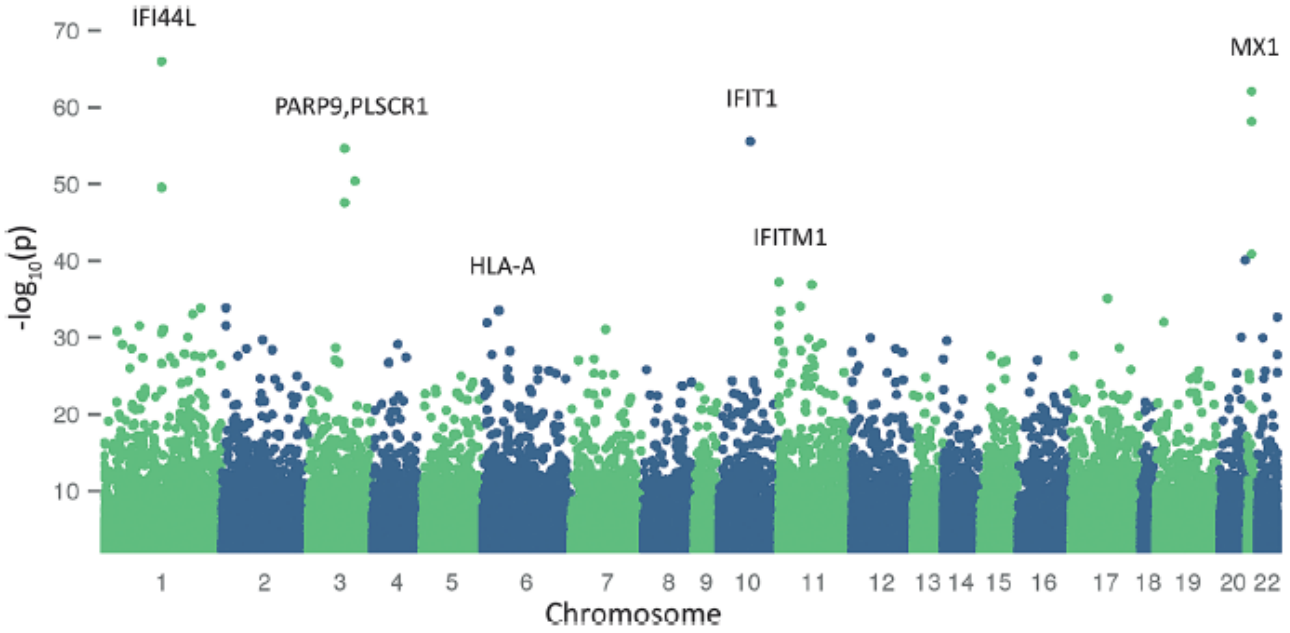
DNA METHYLATION INCREASE WITH DISEASE SEVERITY IN PSS



AND ARE STRONGLY DEPENDENT ON THE PRESENCE OF AUTOANTIBODIES



EWAS IN PSS WHOLE BLOOD



In our study we used a well known reference based method for cell type estimation of whole blood samples.[23 24] In the purified CD19+ B cells compared with the whole blood analysis, we noted a larger mean difference in methylation levels between cases and controls for many associated CpG sites, including at *MX1* and *IFI44L*, perhaps indicating the advantage of a single cell type in the analysis. However, the smaller

Imgenberg-Kreuz et al., ARD, 2016

PATHWAY ANALYSIS

LB : affected / non affected :

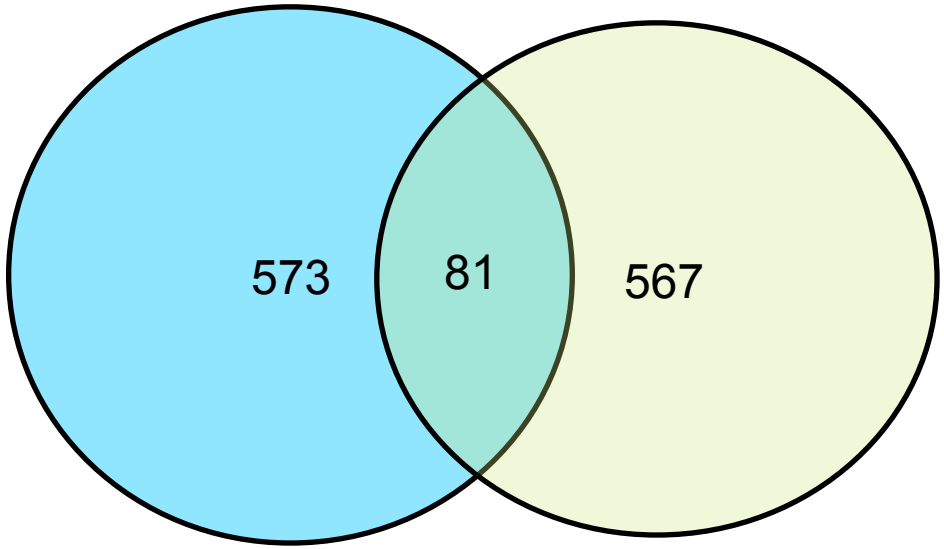
- plasma membrane (228 genes, 23.41%, p-value= 6E-10)
- Systemic autoimmune disease (72 genes, **p-value=2.9E-6)**
- **Rheumatoid arthritis (79 genes, p-value=3.7E-7)**
- **Systemic lupus erythematosus (10 genes, p-value= .5E-6)**
- **Primary Sjögren's syndrome (4 genes, p-value=7.7E-3)**
- Quantity of B lymphocytes (28 genes, p-value=3.4E-5)
- Lymphomagenesis (57 genes, p-value=3.4E-4)
- regulation of apoptosis (51 genes, p-value= 0.001)

LT : affected / non affected :

- membrane (258 genes, pvalue= 4.15E-17)
- immune response (13 genes, p-value= 2.32E-4)
- regulation of apoptosis (39 genes, p-value= 0.0035)

Miceli-Richard et al., ARD, 2015

OVERLAP WITH LUPUS PROVIDES STRONG EVIDENCE FOR A IFN SIGNATURE IN SJOGREN'S



Sjogren's syndrome B-cells

Absher et al., Plos Genet 2013

Strong enrichment of a IFN signature (P<0.0001)
TAP1, *IFITM1*, *IFITM3*, *IFI4L4*, *IRF5*, *PARP12*, *LGALS3BP*, *ITGAX*, *IL6R*….

Predicted upstream regulators include (P<10E-5) :
IFNA2, *IFNy*, *IRF1*, *IRF7*

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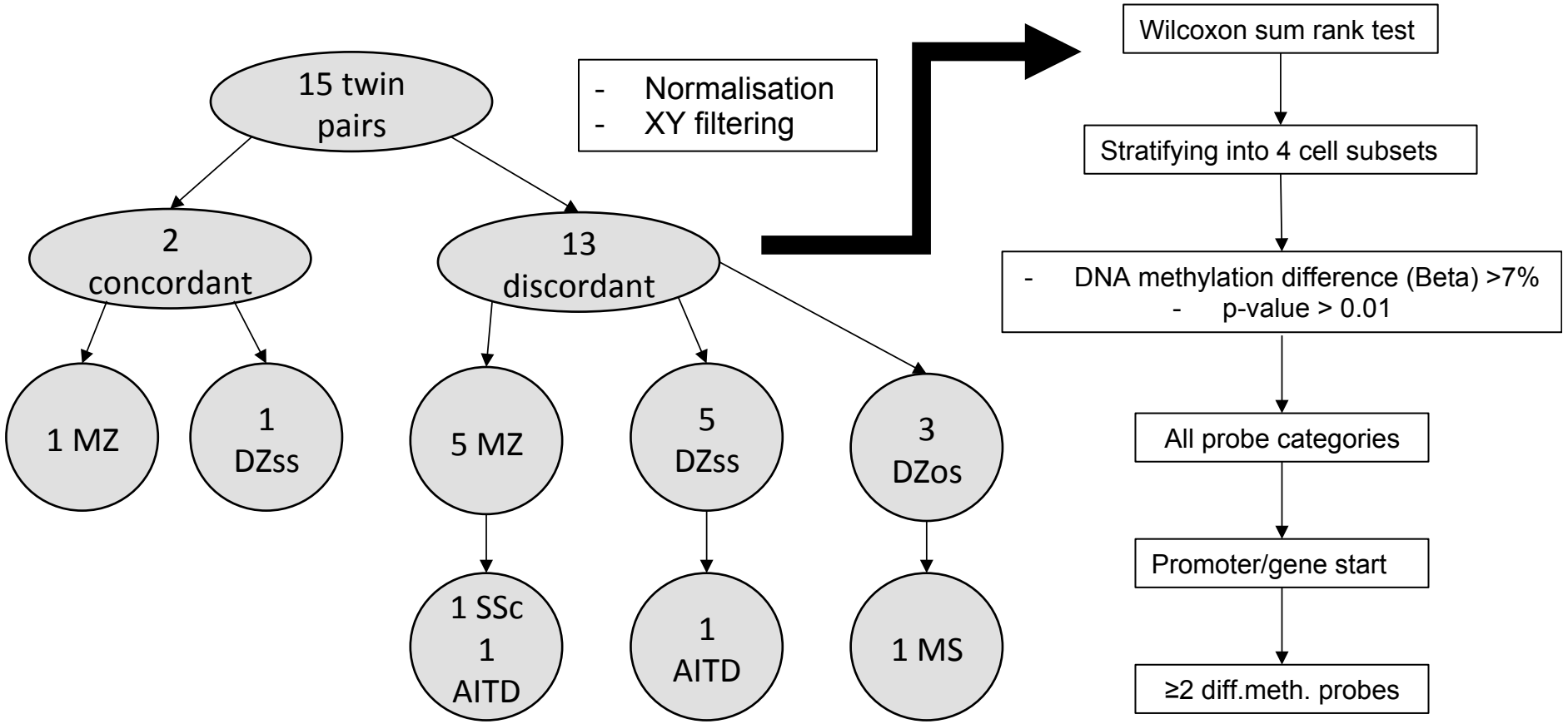
TWISSLE STUDY



Ulff-Møller et al., *Arthrit. Rheumatol.* (2018)

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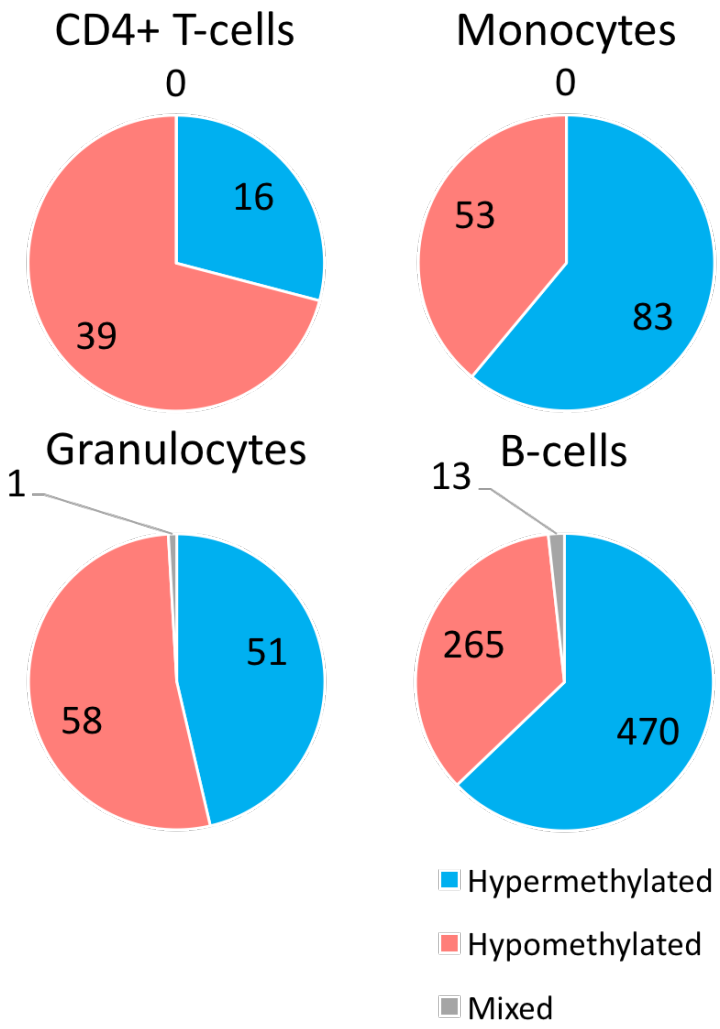
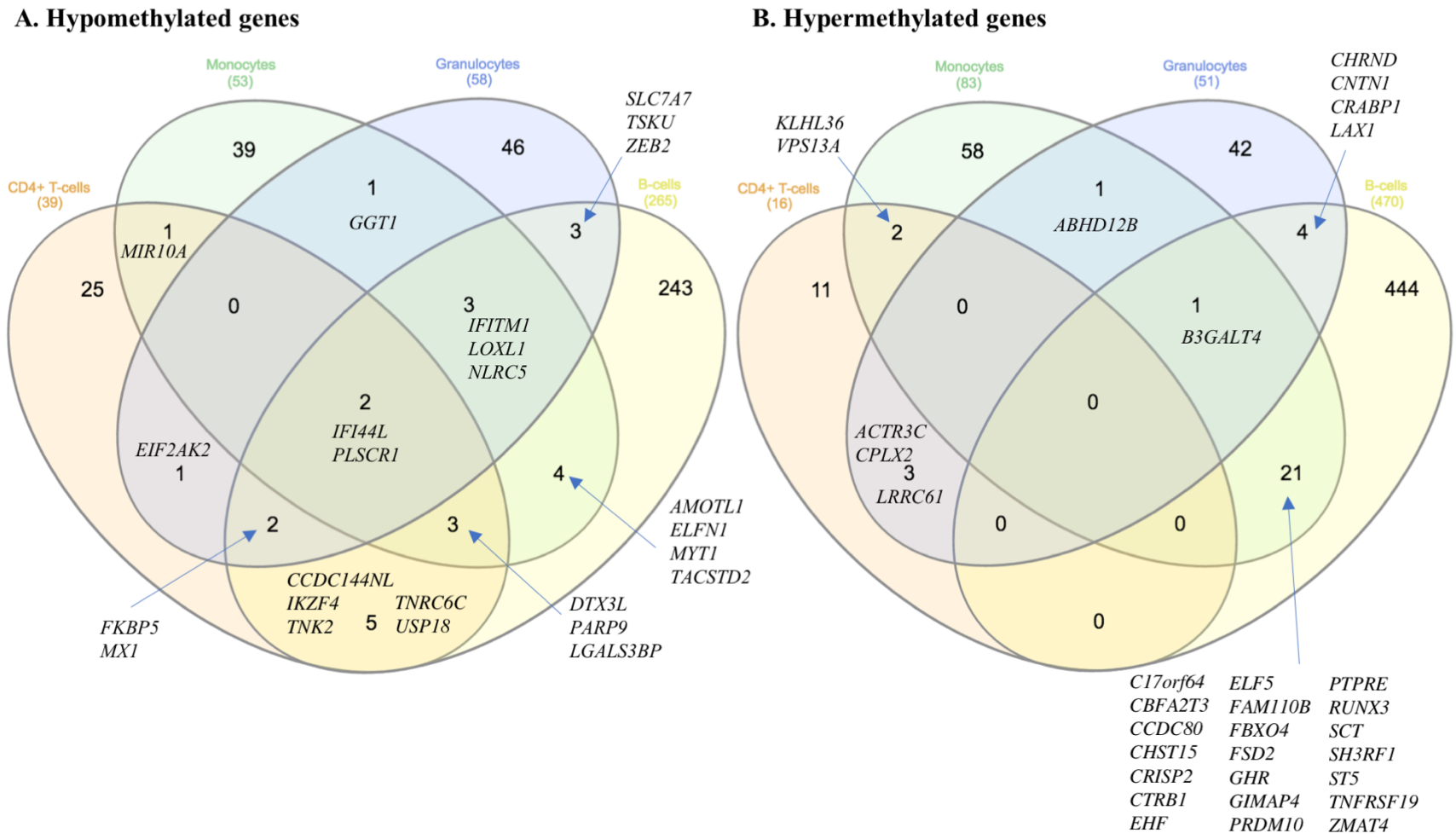
LARGE SCALE DNA METHYLATION CHANGES IN B CELLS OF LUPUS PATIENTS



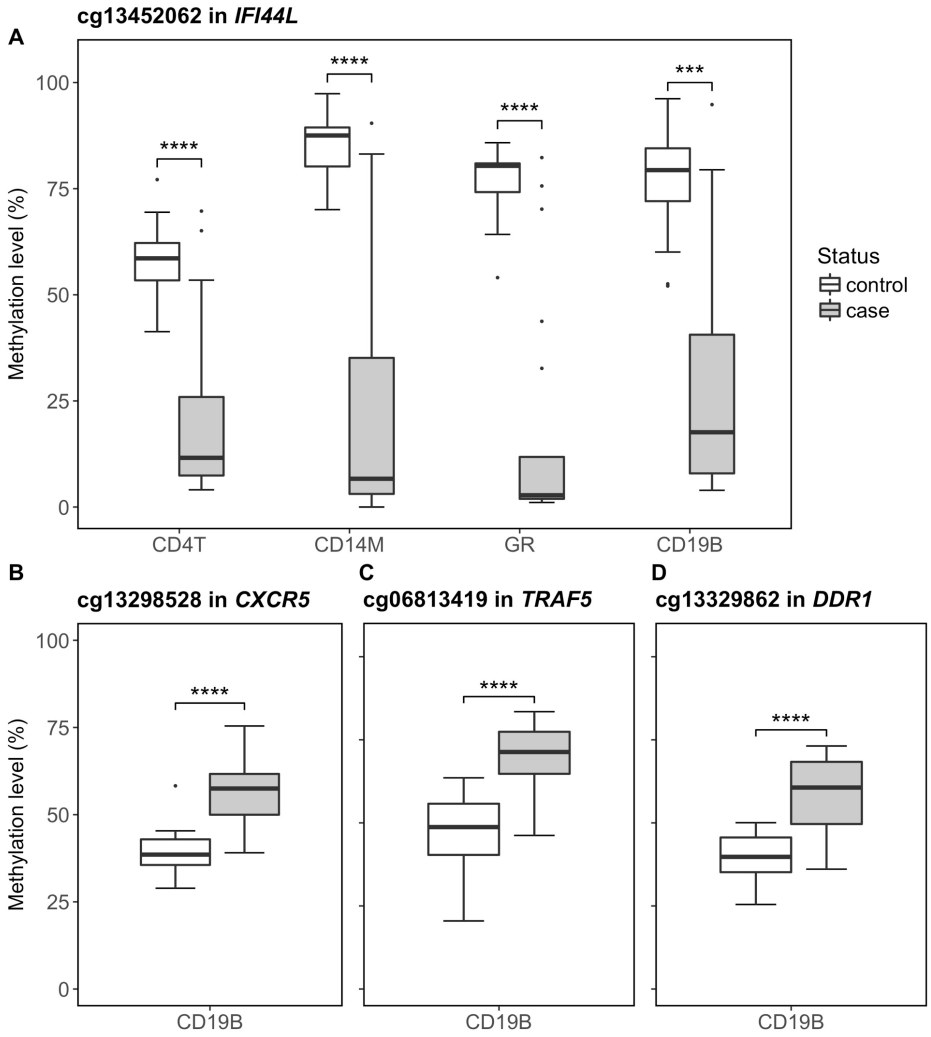
Ulff-Møller et al., *Arthrit. Rheumatol.* (2018)

INTERFERON SIGNATURE IS SHARED BETWEEN DIIFFERENT CELLTYPES



Ulff-Møller et al., *Arthrit. Rheumatol.* (2018)

REPLICATION IN A SMALL CASE / CONTROL STUDY



Ulff-Møller et al., *Arthrit. Rheumatol.* (2018)

LETTER

An epigenome-wide association study of total serum immunoglobulin E concentration

Liming Liang[1], Saffron A. G. Willis-Owen[2]*, Catherine Laprise[3]*, Kenny C. C. Wong[2], Gwyneth A. Davies[4], Thomas J. Hudson[5,6], Aristea Binia[2], Julian M. Hopkin[4], Ivana V. Yang[7], Elin Grundberg[8], Stephan Busche[8], Marie Hudson[9], Lars Rönnblom[10], Tomi M. Pastinen[8,11], David A. Schwartz[7], G. Mark Lathrop[8]§, Miriam F. Moffatt[2]§ & William O. C. M. Cookson[2]§

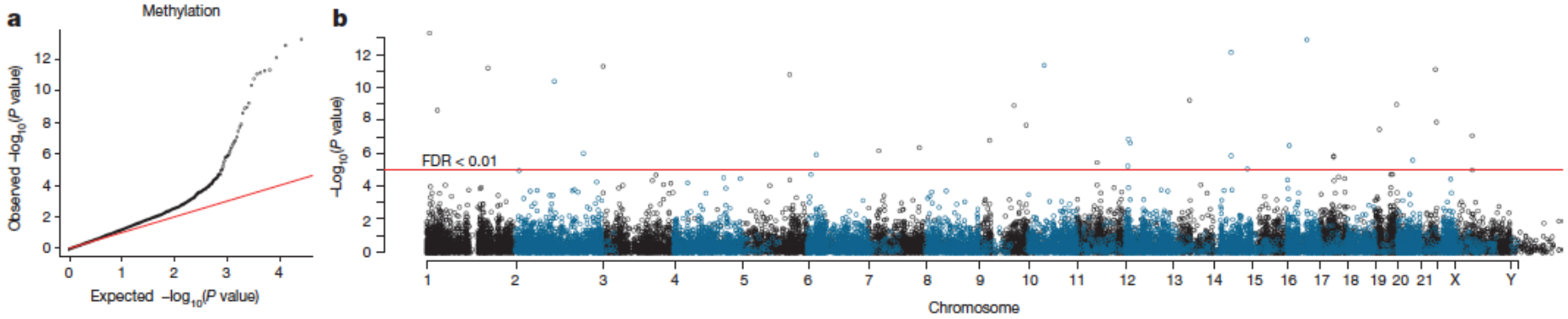


Figure 1 | Manhattan plot of the results of the genome-wide methylation association study. a, b, The results of genome-wide association testing to CGIs are shown for 27,000 loci in 355 subjects from the MRCA panel of families. a, The QQ plot showing observed versus expected $-\log_{10}(P$ values) for association at all loci. b, Manhattan plot showing chromosomal locations of $-\log_{10}(P$ values) for association at each locus. The red line illustrates the threshold for an FDR < 0.01.

EWAS MIGHT EXPLAIN A GREATER % OF PHENOTYPIC VARIABILITY

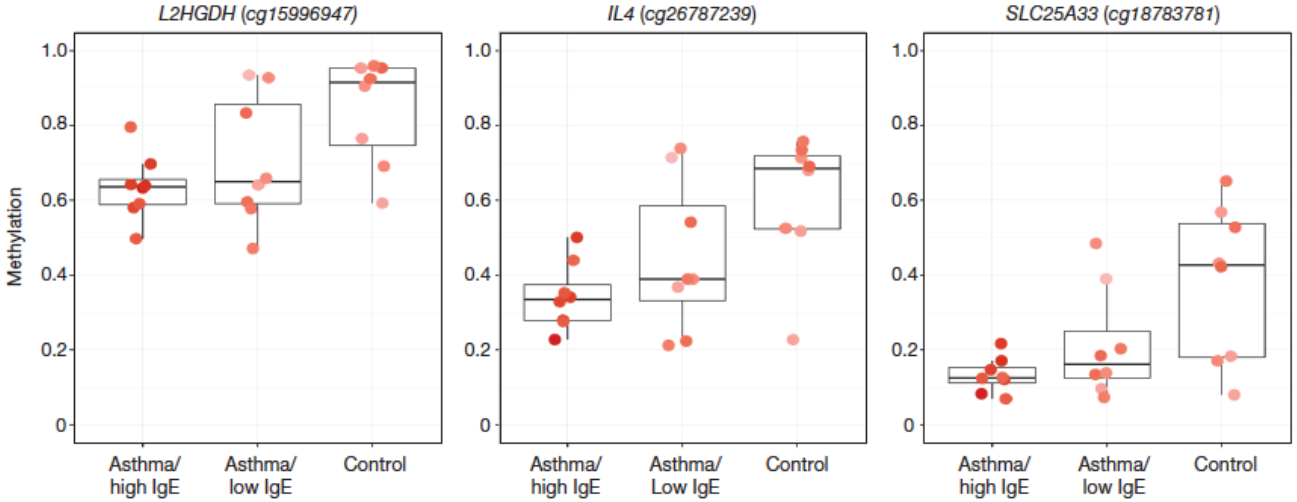
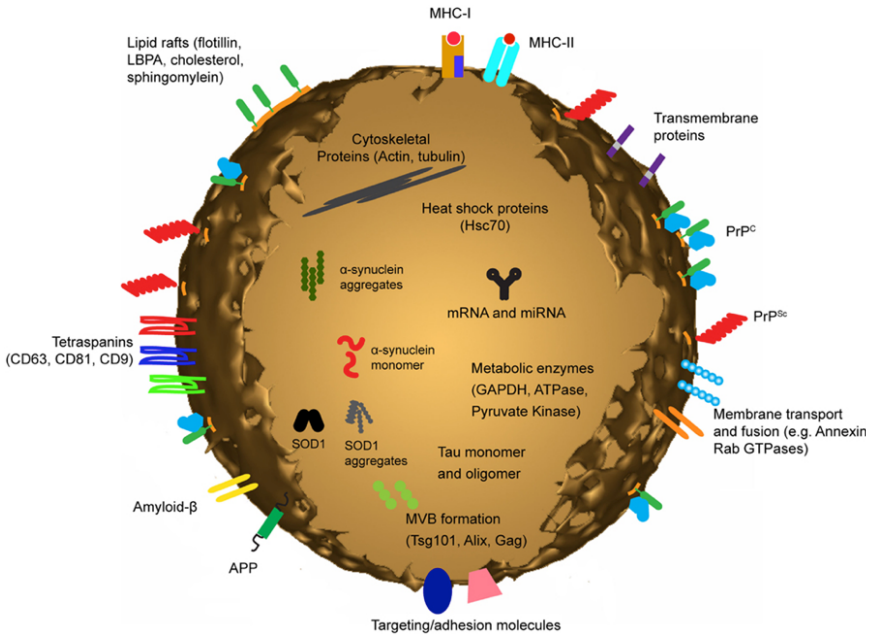


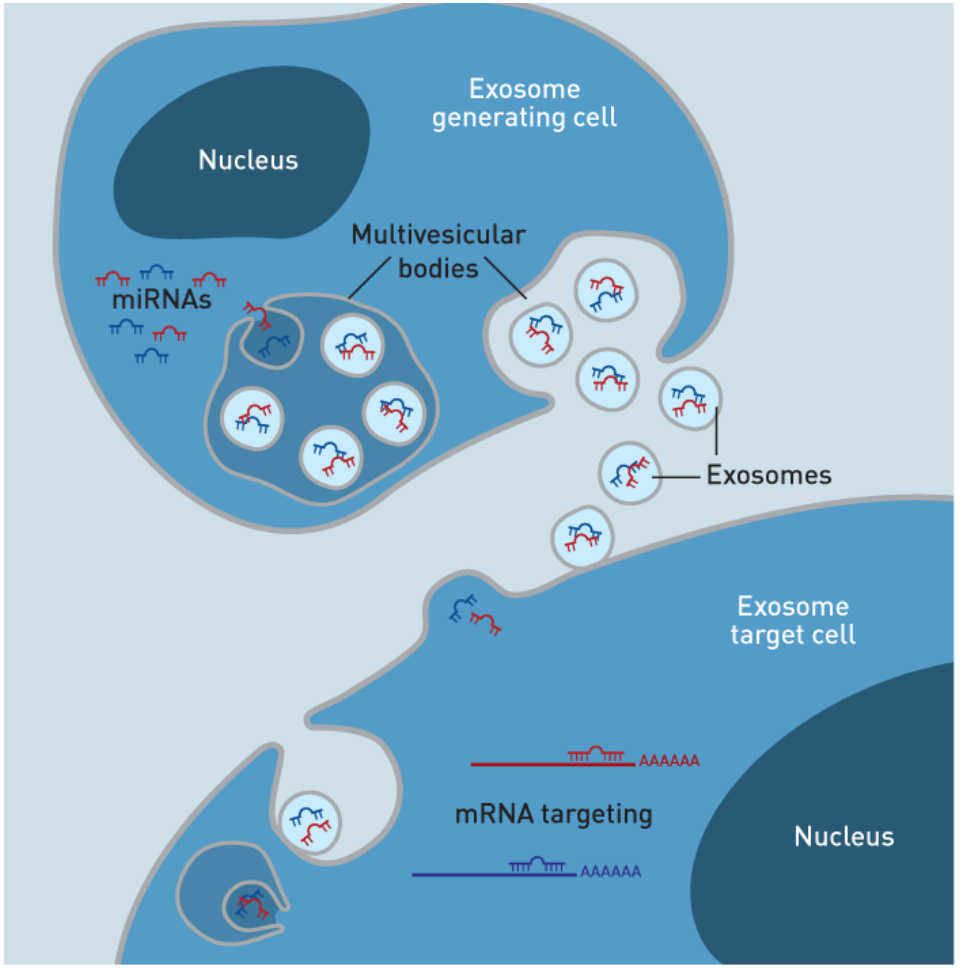
Table 1 | Subject characteristics

	MRCA (discovery)	PAPA (1st replication)	SLSJ (2nd replication)
Number	355	149	160
Age (mean (range))	28 (2–61)	21 (18–30)	29 (5–79)
N (%) female	17 (48.5%)	72 (48.3%)	80 (50.0%)
N (%) asthmatic	175 (49.3%)	34 (22.8%)	69 (43.1%)
N (%) smoker	45 (12.7%)	33 (22.1%)	28 (17.5%)
Eosinophil count (mean ± s.e.m.) per μl	406 ± 383	246 ± 214	242 ± 205
Geometric mean serum IgE (range) (IU l^{-1})†	320 (1–4,999)	663 (0–18,800)	412 (2–7,653)

Our epigenome-wide association study has discovered reproducible CGI associations accounting for a variation in the total serum IgE that is tenfold higher than that derived from large SNP genome-wide association studies[4]. In contrast to SNP studies, association to methylation

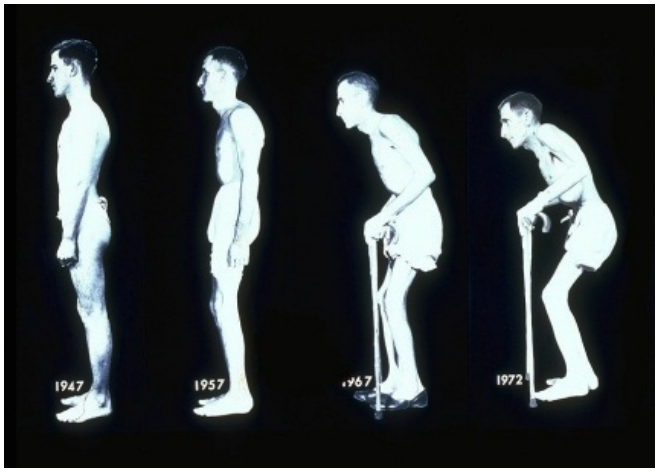
EXOSOMES – VEHICLES OF INTERCELLULAR SIGNALLING

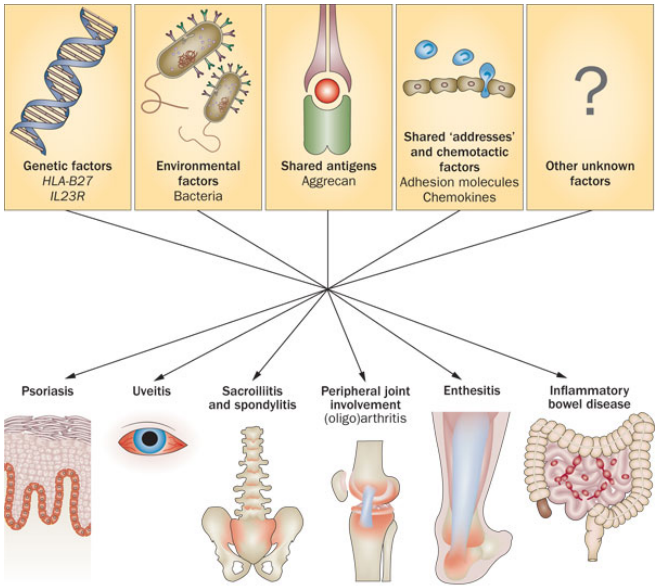




Source: EXIQON website Oct 2016

ETIOLOGY OF SPONDYLOARTHRITIS





Rosenbaum. & Rosenzweig Nat. Rev. Rheumatol (2012)

- Family of inflammatory rheumatisms (axial spondyloarthritis, psoriatic arthritis, IBD related arthritis and reactive arthritis)
- Very limited understanding of the pathogenesis
- Susceptibility attributable to genetic factors is 80 - 90% (twin studies)
- HLA B27: strong association but does not explain all genetic susceptibility
- Genome-wide association studies:
  - *IL23R*
  - *IL1R2*
  - *RUNX3*
  - *CARD9*
  - *IL12B*
  - *IL6R*
  - *ERAP1*
  - *ANTXR2 …*

Tost, Miceli-Richard, Dougados, unpublished

SMALLRNA SEQUENCING (HISEQ 4000)

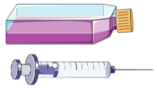
Biological sources

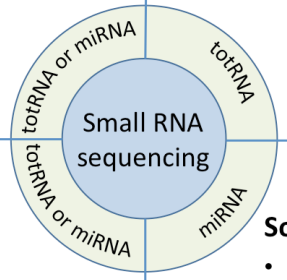
High quality samples
- Blood
- Cell lines
- Biopsy

Plasma & serum
- Fresh or frozen fraction
- Low volumes: 0.5 to 1.5mL

⇩

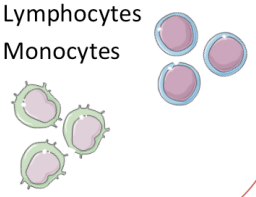
Exosomes

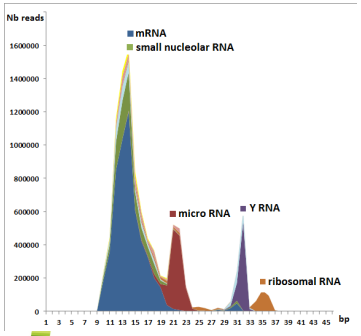




Low quality samples
- Frozen tissues
- FFPE (Formalin-Fixed Paraffin-Embedded)

Sorted immune cells :
- Lymphocytes
- Monocytes





miRNA Analysis pipeline

Quality Control	Trimming	Mapping miRBase	miRNA Counts	Differential Analysis
fastqc (0.11.3)	trimmomatic (0.32)	bowtie (1.1.2)	samtools (0.1.19)	DESeq2 (1.6.3)

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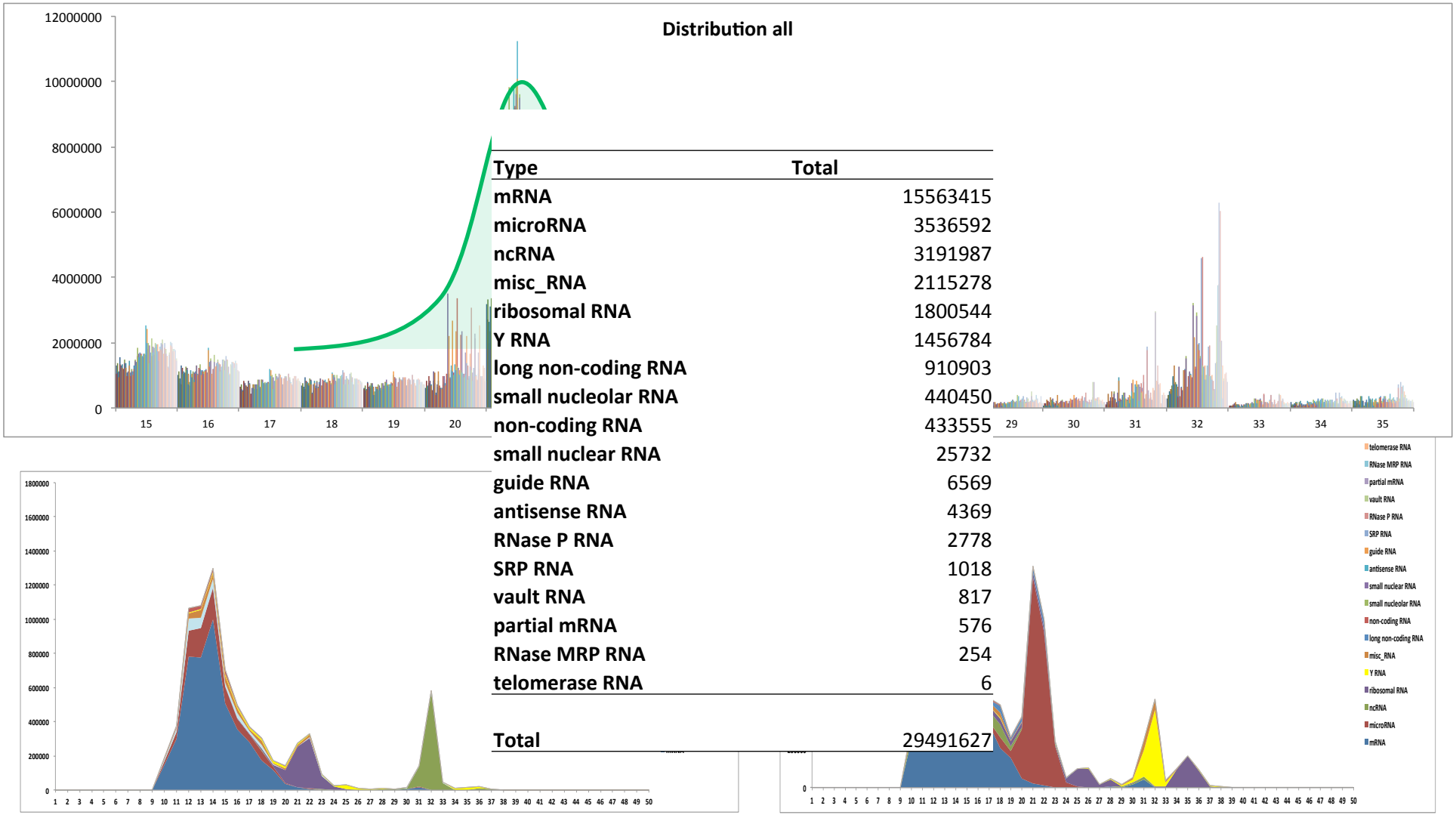
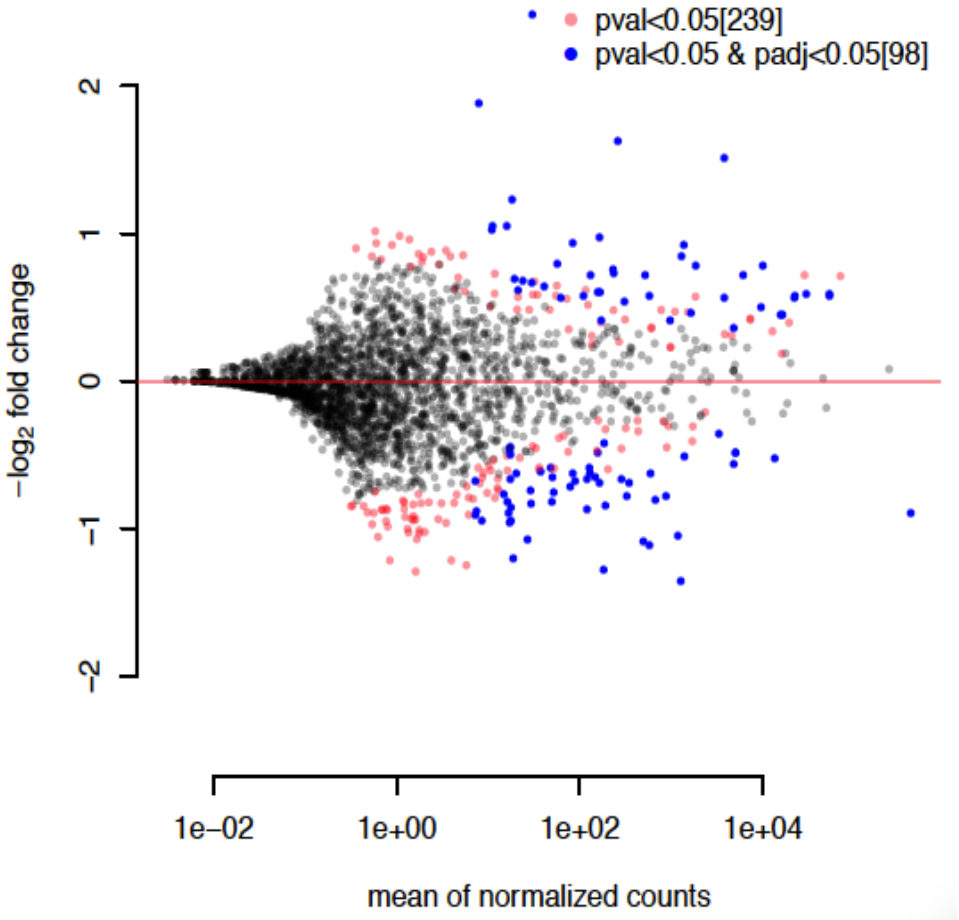
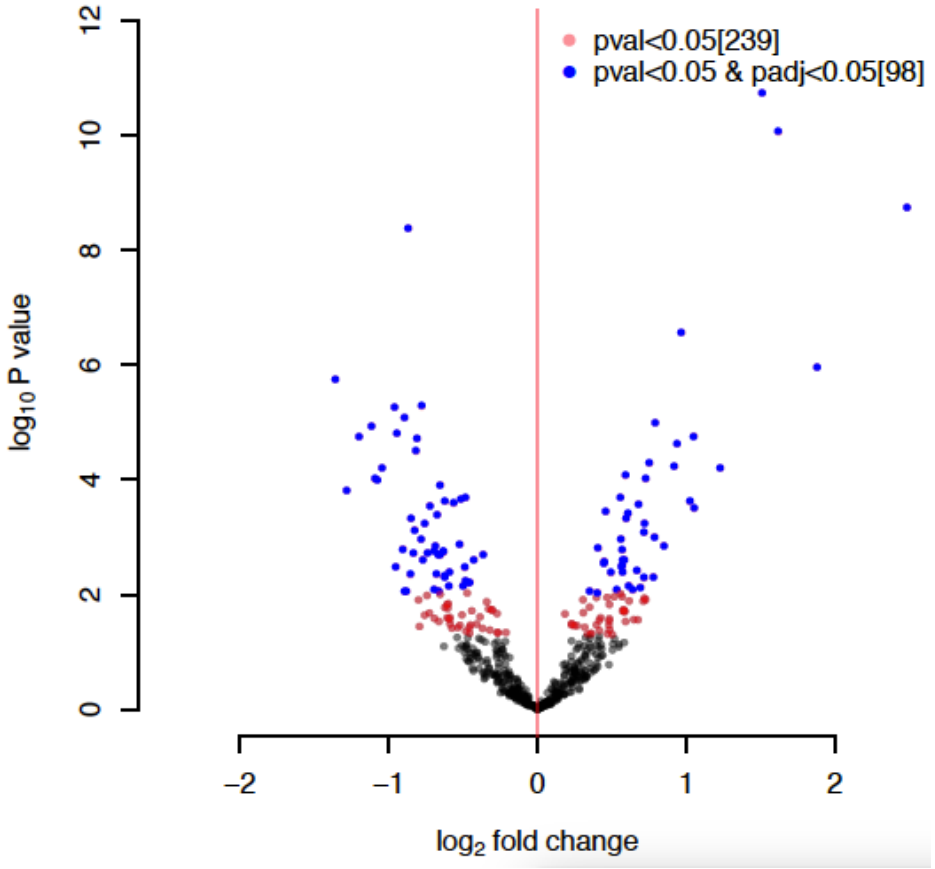
RNA CONTENT OF EXOSOMES



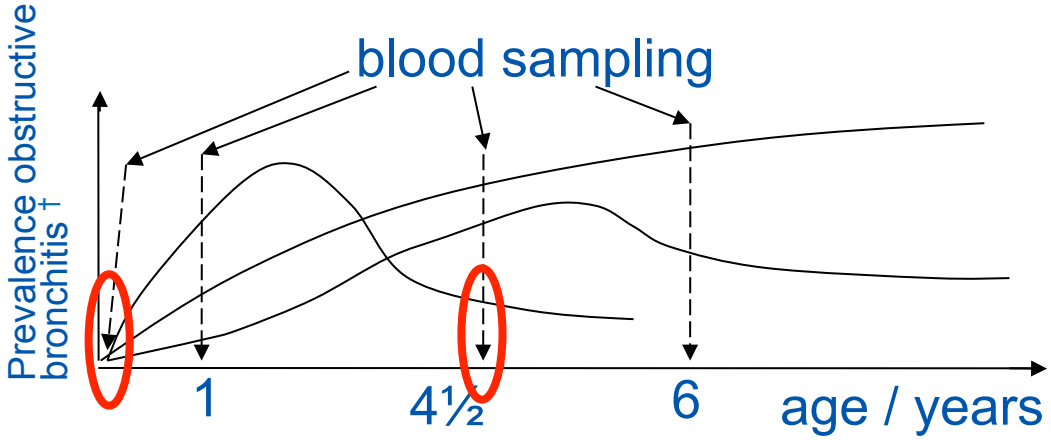
Type	Total
mRNA	15563415
microRNA	3536592
ncRNA	3191987
misc_RNA	2115278
ribosomal RNA	1800544
Y RNA	1456784
long non-coding RNA	910903
small nucleolar RNA	440450
non-coding RNA	433555
small nuclear RNA	25732
guide RNA	6569
antisense RNA	4369
RNase P RNA	2778
SRP RNA	1018
vault RNA	817
partial mRNA	576
RNase MRP RNA	254
telomerase RNA	6
Total	29491627

DIFF EXPRESSED MIRNAS IN EXOSOMES IN PATIENTS WITH SPONDYLOARTHRITIS





Epigenetic effects on asthma in a rural environment (PASTURE)



- Longitudinal study following children in rural areas of Germany, Switzerland, Austria, France and Finland with mothers living on a farm during pregnancy and matched controls
- Focus on asthma and allergic diseases
- Cord blood and PBMCs at 1, 3, 4.5 and 6 years

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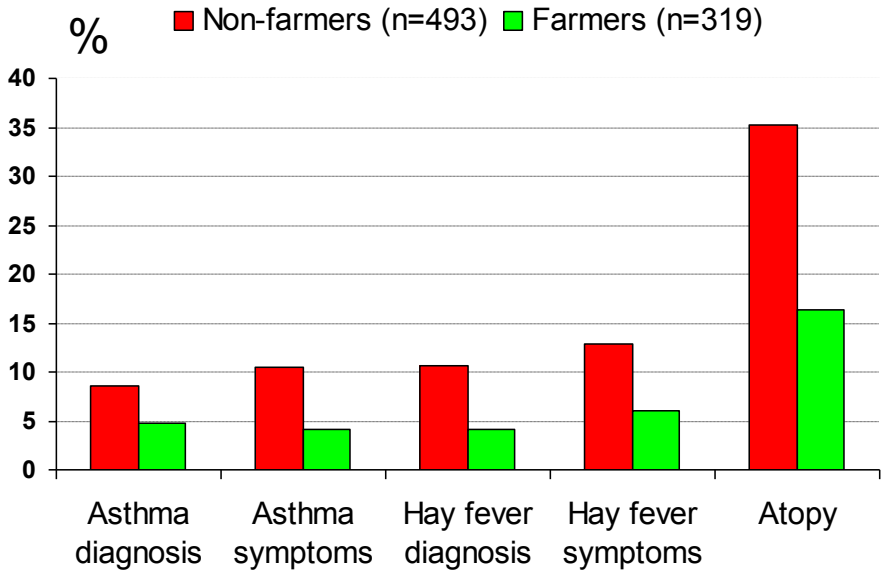
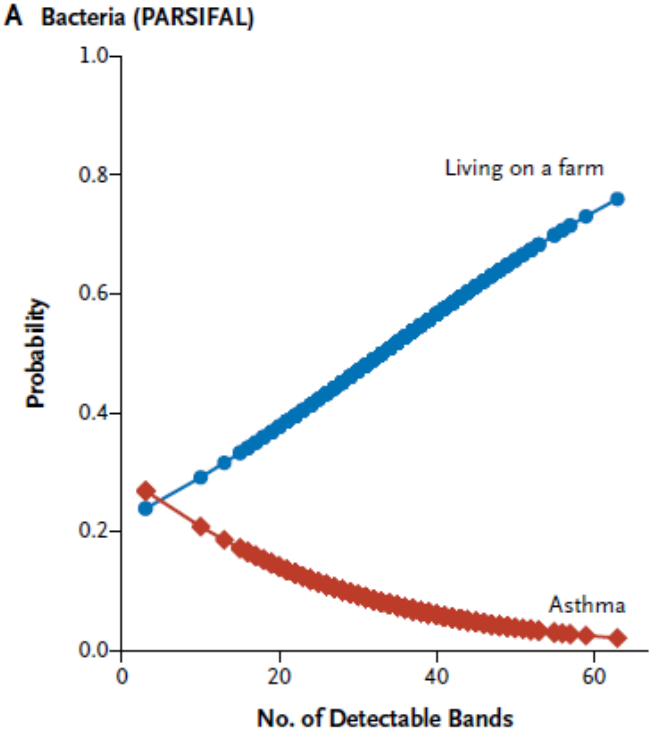
† *Stern et al., 2006; Michel et al. Allergy 2013*

FARM EXPOSURE PROTECTS FROM ASTHMA AND ATOPY

Lower prevalence of asthma, hay fever and atopy in farm children†

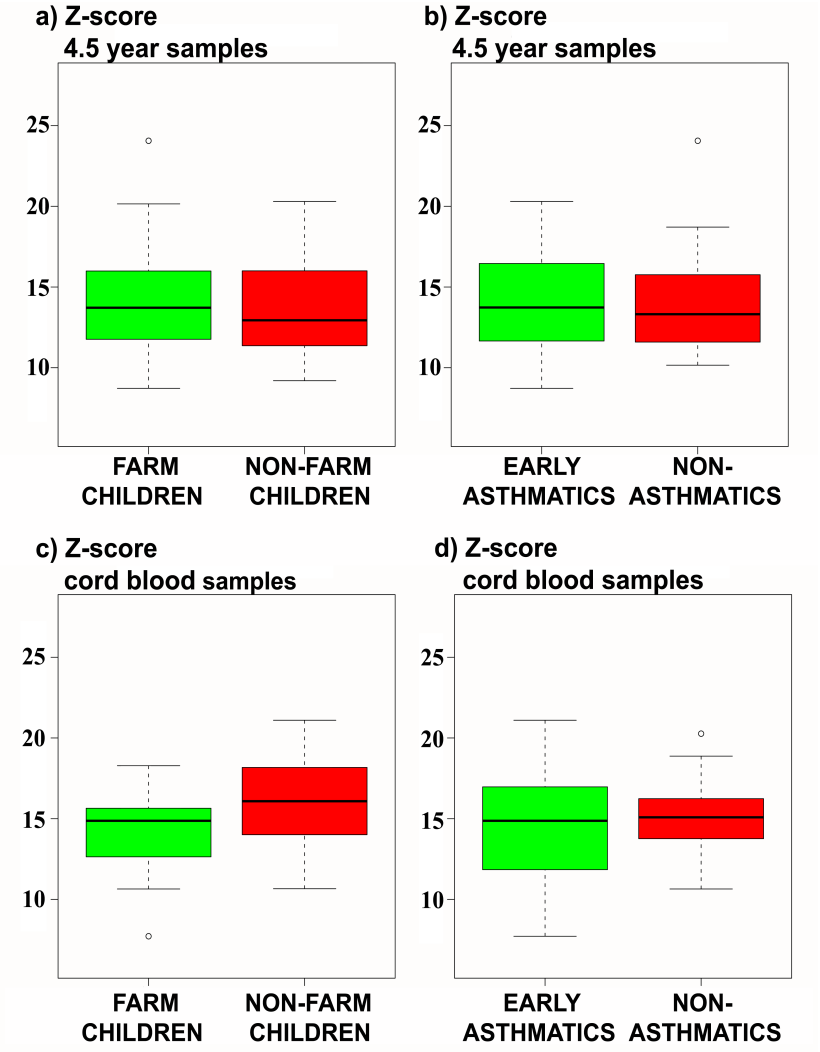


Microbial diversity and asthma are reversely related*



† Riedler et al. Lancet, 2001, * Ege et al, N Engl J Med, 2011

FARM ENVIRONMENT INDUCES A DIFFERENT EPIGENETIC BASE LINE



Michel et al., Allergy, 2013

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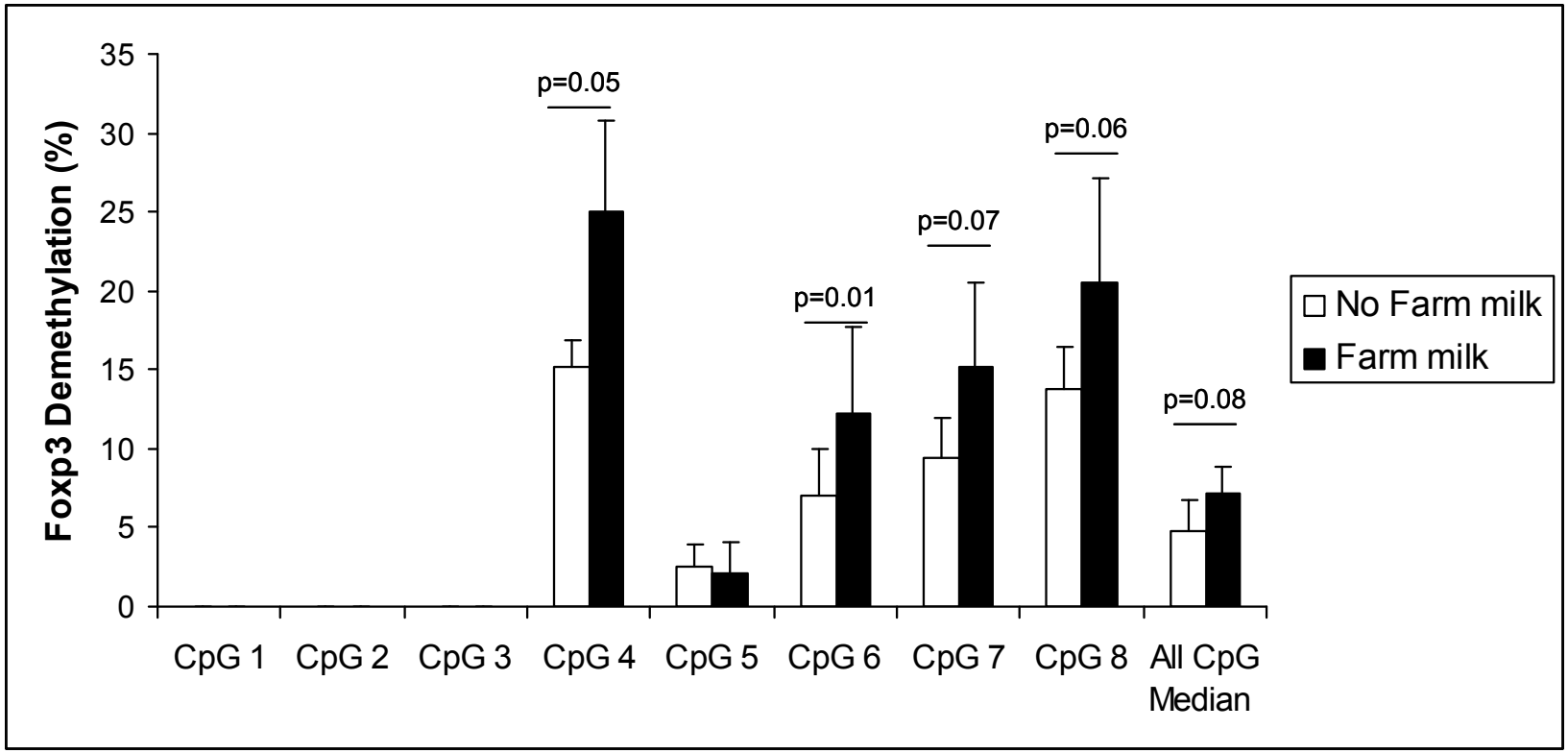
TREND FOR HIGHER DEMETHYLATION IN THE TREG MASTER GENE *FOXP3* AFTER FARM MILK EXPOSURE



Lluis et al., JACI, 2013

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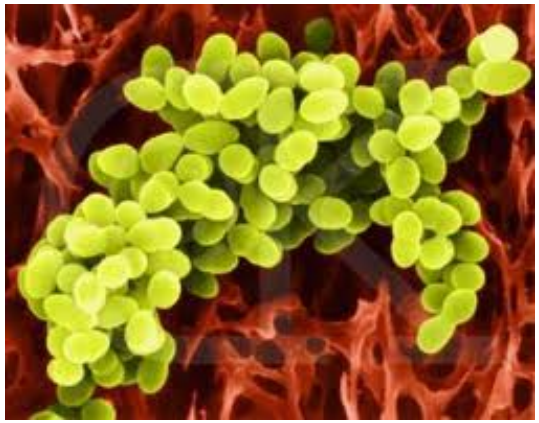
ALLERGY PROTECTIVE EFFECTS OF BACTERIA ISOLATED FROM COW SHEDS

Acinobacter Lwoffi

Lactococcus Lactis



PRENATAL MICROBIAL (*ACINOBACTER LWOFFII*) EXPOSURE HAS ASTHMA PROTECTIVE EFFECTS IN MICE

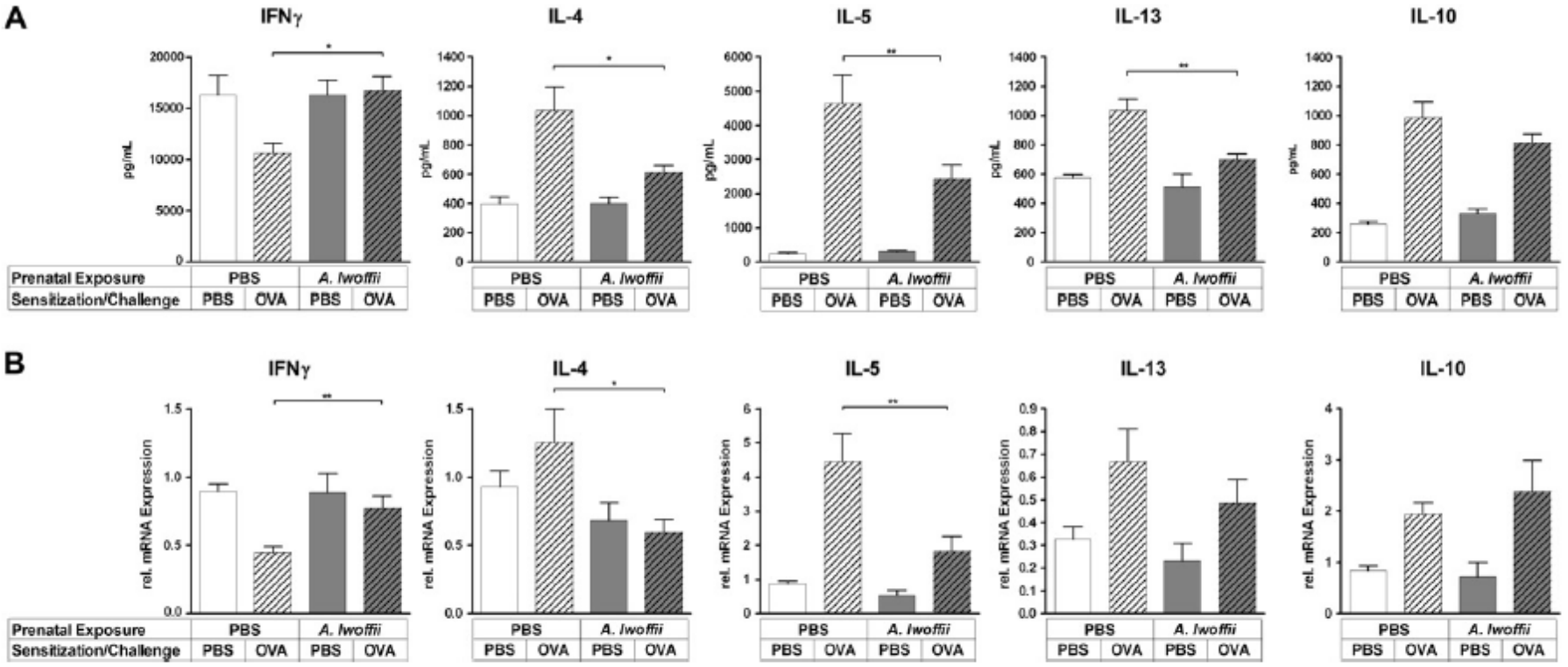
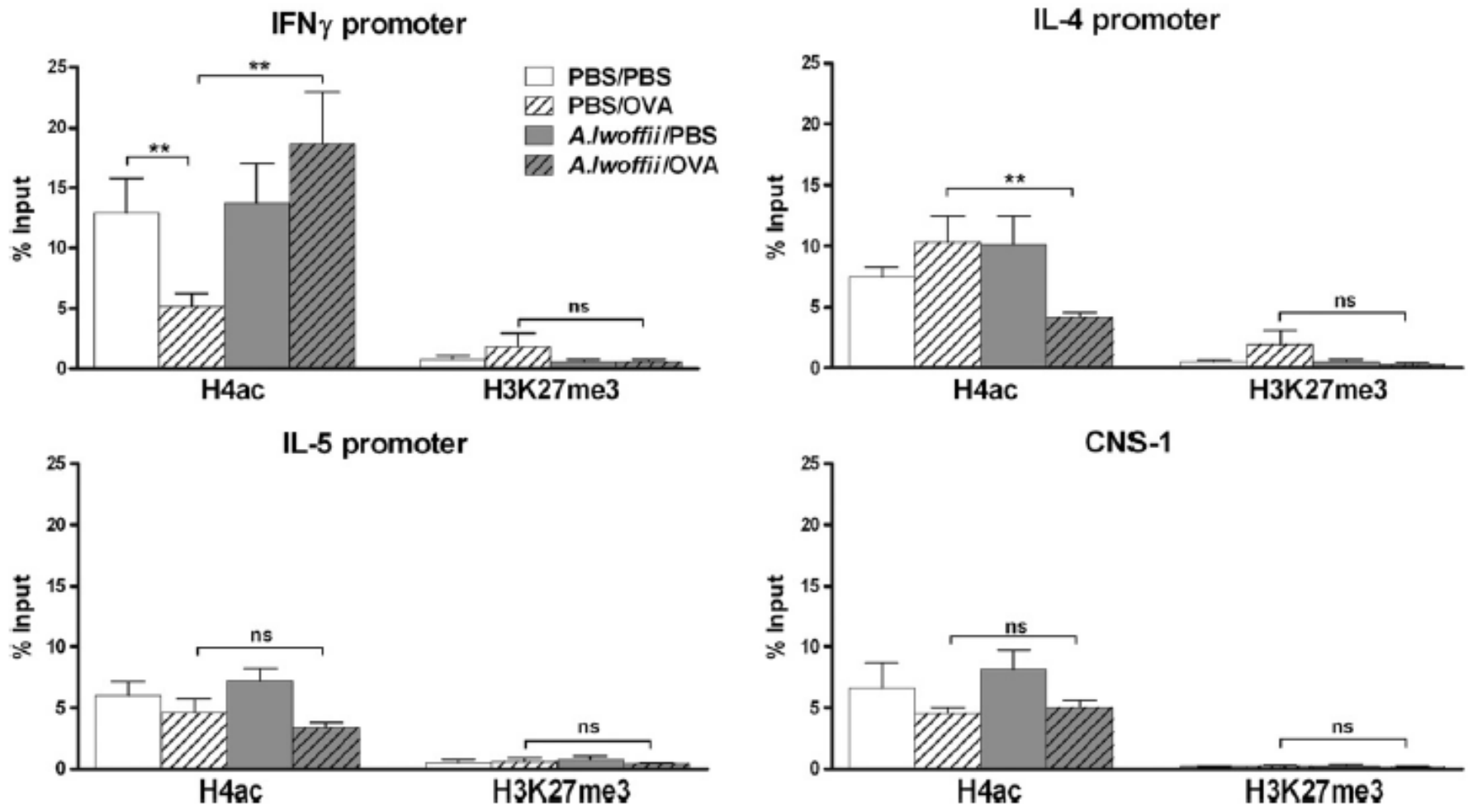


FIG 1. T_H1/T_H2 cytokine balance after prenatal *A lwoffii* F78 exposure. **A**, Concentrations of IFN-γ, IL-4, IL-5, IL-13, and IL-10 in the supernatant of anti-CD3/anti-CD28–stimulated mononuclear spleen cells from offspring from *A lwoffii* F78– or sham-exposed (PBS) mothers. **B**, Relative mRNA expression of IFN-γ, IL-4, IL-5, IL-13, and IL-10 in $CD4^+CD25^-$ splenic T cells. Data represent the results of 2 independent experiments, each with 4 to 8 individually analyzed animals per group. $*P < .05$, $**P < .01$.

Brand et al., JACI, 2011.

TH1/TH2 BALANCE ALTERED THROUGH MODIFICATION OF THE HISTONE MODIFICATION PROFILE



Brand et al., JACI, 2011.

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GENERAL CONCLUSIONS

- Genetics and epigenetics yield complementary information, but contribution of each molecular level might differ in function of the investigated question.
- Technologies have matured very recently allowing genome-wide and whole-genome epigenetic studies as well as reproducible and FDA approved diagnostic assays
- Epigenetic patterns are tissue-specific requiring cell sorting or enrichment to decipher the more subtle changes in complex diseases
- Autoimmune diseases display common themes, but also disease specific changes and different cell types might be the most important ones
- Epigenetic alterations are widespread in different complex diseases, but at lower amplitude and might be used for early detection and clinical management

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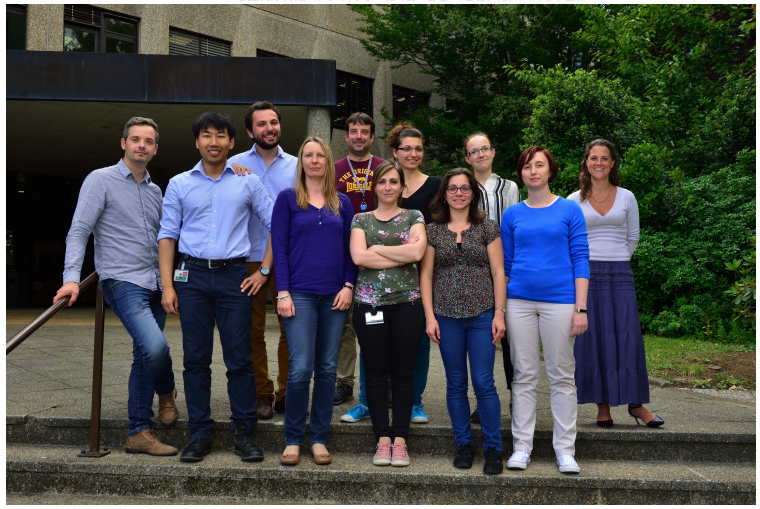

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